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# United States Patent [19]

Lastick et al.

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[54] COMBINED ENZYME MEDIATED FERMENTATION OF CELLULOUS AND XYLOSE TO ETHANOL BY *SCHIZOSACCHAROMYCES POMBE*, CELLULASE,  $\beta$ -GLUCOSIDASE, AND XYLOSE ISOMERASE

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[73] Assignee: The United States of America as represented by the United States Department of Energy, Washington, D.C.

[21] Appl. No.: 28,592

[22] Filed: Mar. 8, 1993

## Related U.S. Application Data

[63] Continuation of Ser. No. 672,984, Mar. 21, 1991, abandoned.

[51] Int. Cl.<sup>5</sup> ..... C12P 7/12

[52] U.S. Cl. ..... 435/165; 435/161;

435/162; 435/163; 435/171; 435/255.1

[58] Field of Search ..... 426/11, 18, 20, 21; 435/161, 163, 165, 162

## References Cited

### U.S. PATENT DOCUMENTS

3,990,944 11/1976 Gauss et al. ..... 435/165  
4,009,075 2/1977 Hoge ..... 435/165  
4,326,032 4/1982 Grove ..... 435/163  
4,326,036 4/1982 Hayes ..... 435/163  
4,368,268 1/1983 Gong ..... 435/161

4,472,501 9/1984 Takasawa et al. ..... 435/163  
4,490,468 12/1984 Gong et al. ..... 435/161  
4,511,656 4/1985 Gong ..... 435/161  
4,628,029 12/1986 Evel Leigh et al. ..... 435/165  
4,663,284 5/1987 Jeffries ..... 435/161  
4,840,903 6/1989 Wu ..... 435/165

## OTHER PUBLICATIONS

Motoo, A. et al., "Conversion of rice straw to ethanol by simultaneous saccharification and fermentation", Chemical Abstracts 103 (23), 19492 6v, 1985.

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## [57] ABSTRACT

A process for producing ethanol from mixed sugar streams from pretreated biomass comprising xylose and cellulose using enzymes to convert these substrates to fermentable sugars; selecting and isolating a yeast *Schizosaccharomyces pombe* ATCC No. 2476, having the ability to ferment these sugars as they are being formed to produce ethanol; loading the substrates with the fermentation mix composed of yeast, enzymes and substrates; fermenting the loaded substrates and enzymes under anaerobic conditions at a pH range of between about 5.0 to about 6.0 and at a temperature range of between about 35° C. to about 40° C. until the fermentation is completed, the xylose being isomerized to xylulose, the cellulose being converted to glucose, and these sugars being concurrently converted to ethanol by yeast through means of the anaerobic fermentation; and recovering the ethanol.

4 Claims, 1 Drawing Sheet

# ARCHIVAL FILE

B02203

Biofuels Information Center

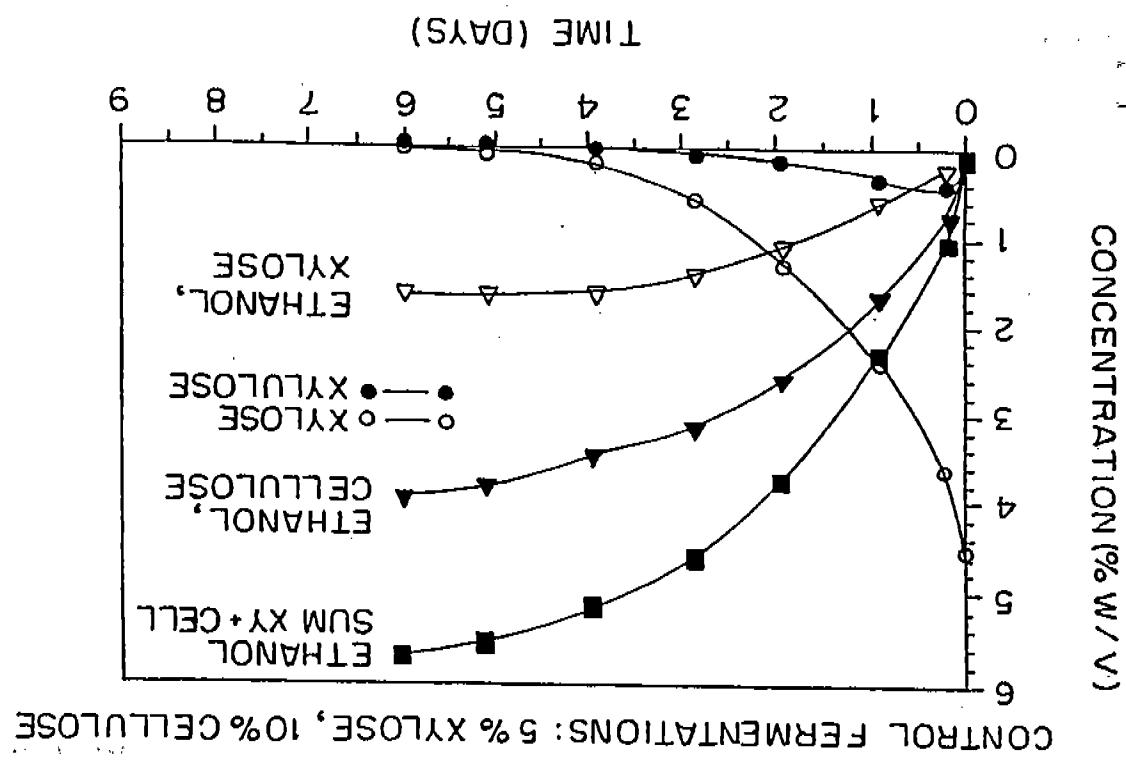


FIG. 2

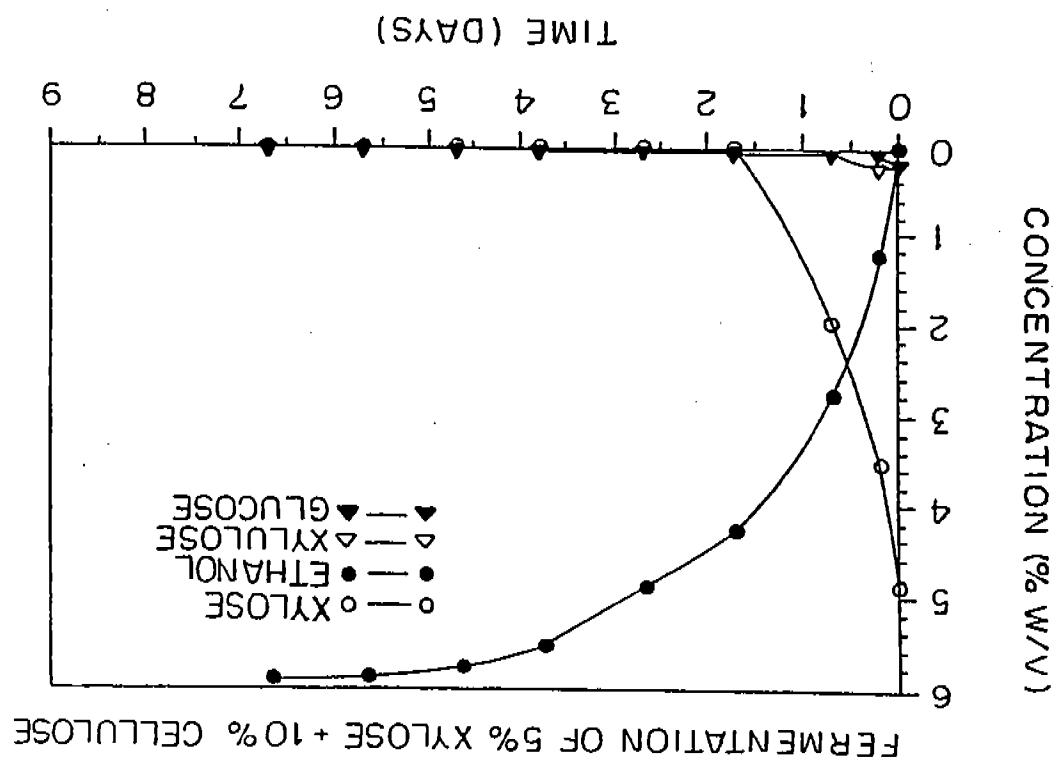


FIG. 1

5,372,939

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U.S. Patent

COMBINED ENZYME MEDIATED  
FERMENTATION OF CELLULOUS AND XYLOSE  
TO ETHANOL BY *SCHIZOSACCHAROYCES*  
*POMBE*, CELLULASE,  $\beta$ -GLUCOSIDASE, AND  
XYLOSE ISOMERASE

CONTRACTURAL ORIGIN OF THE  
INVENTION

The U.S. Government has rights in this invention under Contract No. DE-AC02-83CH10093 between the U.S. Department of Energy and the Solar Energy Research Institute, a Division of Midwest Research Institute.

This is a continuation of application Ser. No. 672,984 filed Mar. 21, 1991 abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to a process that combines a simultaneous saccharification and fermentation (SSF) process and a simultaneous fermentation and isomerization of xylose (SFIx) process to provide a simpler and reduced cost process for producing ethanol. In particular, the invention pertains to an improved process for producing ethanol from a mixed stream of xylose and cellulose and includes fermenting the mixed stream using a cellulase and xylose isomerase enzyme, under predetermined conditions, and includes simultaneously saccharifying the cellulose while fermenting the soluble sugars produced, and concurrently isomerizing the xylose while fermenting the xylulose as it is produced.

2. Description of the Prior Art

U.S. Pat. No. 4,663,284 to Jeffries discloses a process for producing ethanol from D-xylose by fermentation with xylose metabolizing yeasts, wherein small quantities of glucose are added to the fermentation medium during the fermentation process; however, the process is not an enzyme mediated process. Yeast strains can ferment xylose if oxygen is allowed to be present in the fermentation. The process of Jeffries further disclose that the addition of glucose to these oxygen mediated fermentations improves the yield of the fermentation; however, cellulose fermentation is not taught or included.

U.S. Pat. No. 4,511,656 to Gong pertains to a method for producing ethanol directly from D-xylose through fermentation of D-xylose by yeast mutants. The process further provides for directly and simultaneously obtaining ethanol from a mixture of cellulose and hemicellulose through yeast fermentation of D-glucose and D-xylose; however, these are oxygen mediated fermentations of xylose and are supplemented by the addition of glucose, as the sugar or as a hydrolysate containing glucose. In addition to oxygen being required, no enzymes are used and cellulose is not fermented.

In U.S. Pat. No. 4,490,468 to Gong et al., there is described an anaerobic fermentation of xylulose previously obtained by isomerization of xylose, and example 6 thereof briefly mentions the possibility of simultaneous isomerization and fermentation of xylose; however, the process is not combined in any way with the fermentation of cellulose.

U.S. Pat. No. 4,368,268 to Gong relates to a process for the production of ethanol from xylulose. The process includes isomerizing the xylose to xylulose and fermenting the xylulose to ethanol. Essentially, this process is the fermentation of xylose or xylose and other

sugars in hemicellulose hydrolysates by mutant strains of yeast, either aerobically or anaerobically; however, hemicellulose does not refer to cellulose but to extracts obtained by pretreatment of materials that contain cellulose. The sugars obtained are soluble sugars (in most cases mostly xylose). Cellulose is not soluble and must be enzymatically digested to produce soluble sugars. Further, fermentations of xylose or hemicellulose, whether or not the hemicellulose contains some glucose, is not cellulose fermentation, and the fermentations in this patent are not anaerobic but oxygen mediated rather than enzyme mediated fermentations.

U.S. Pat. No. 4,840,903 to Wu discloses a process for the production of ethanol by a fungal strain capable of slowly degrading and fermenting cellulose, xylose, and a number of other sugars. Like simultaneous saccharification and fermentation (SSF) of cellulose, cellulase enzymes were added to the fermentations to produce glucose from cellulose; however, the fermentations are not a combination of enzymatic isomerization of xylose to xylulose a fungal strain rather than ethanol tolerant yeast was used for the fermentation, and fungal strains take much longer to grow and ferment, and these longer lengths of time or slow rates are unacceptable for industrial purposes.

In biomass materials, cellulose and hemicellulose are the two most abundant and renewable raw organic compounds, and together they compose above 70 percent of the entire world's plant biomass on a dry weight basis. These raw materials are widely available in waste from agricultural, forest, vegetable and food process sources, and the efficient recycling of these wastes to useful products, such as ethanol, would help to reduce disposal problems as well as provide an abundant and cheap source of fuel.

SUMMARY OF THE INVENTION

It is an object of the invention to produce ethanol from xylose using cellulosic biomass waste without having to ferment separately a stream containing soluble xylose in an enzyme mediated process.

A further object of the invention is to produce ethanol from xylose using cellulosic biomass waste without having to separately ferment the cellulose in the feedstock.

A yet further object of the invention is to produce ethanol using cellulosic biomass waste by combining a simultaneous fermentation and isomerization of xylose (SFIx) process and a simultaneous saccharification and fermentation (SSF) process to simplify the entire biomass to ethanol process and to significantly reduce costs because of significantly reduced equipment requirements.

In general, the objectives of the invention are accomplished by producing ethanol from a mixed stream of xylose and cellulose using enzymes to convert these carbohydrates to fermentable sugars under predetermined conditions. This is done by the simultaneous conversion of cellulose to glucose, using cellulase enzymes, and the conversion of xylose to xylulose, using the enzyme xylose isomerase in the presence of *Schizosaccharomyces pombe* ATCC No. 2476. The enzymatic processes allow for these fermentable sugars, glucose and xylulose, to be converted by yeast to ethanol in the same fermentation. The continuous conversion of the sugars to ethanol by the fermenting yeast is key to the process because the activities of the enzymes are inhib-

DETAILED DESCRIPTION OF THE  
INVENTION

FIG. 1 depicts the yield of ethanol from fermentation of 10% (w/v) cellulose and 5% (w/v) xylose performed at 37°C. At pH values of 5.5 to 6 using *Schizosaccharomyces pombe* ATCC 2476 (NRRL Y-164) and commercial yeast (Genencor 2000 IFPU/L),  $\beta$ -glucosidase alone using the simultaneous fermentation method resulted in a 50% increase in ethanol yield compared to the separate fermentation of cellulose and xylose. The simultaneous fermentation method also resulted in a 20% increase in ethanol yield compared to the separate fermentation of cellulose and xylose.

## BRIEF DESCRIPTION OF THE DRAWINGS

Indeed in the presence of ether products, glucose (and the disaccharide cellulose) and xylose.

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